THE UNIVERSITY OF ALBERTA

COCCIDIA OF CALIFORNIA QUAIL IN THE OKANAGAN VALLEY, BRITISH COLUMBIA

by


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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Coccidia of California Quail in the Okanagan Valley, British Columbia" submitted by Eugene McLaren Liburd in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

During the summers of 1965 and 1966 a total of 85 California quail (*Lophortyx californicus*) were collected by trapping or shooting from four study areas in the southern Okanagan Valley, British Columbia, and examined for oocysts of *Eimeria* (Protozoa:Eimeriidae). Oocysts belonging to two species of *Eimeria* were found in the faeces, intestinal and/or caecal contents of 73% of the quail examined. These are temporarily labeled as *Eimeria* sp. "A" and *Eimeria* sp. "B" pending further studies on their life cycles. Oocysts of *Eimeria* sp. "A" measured 20.60-26.30 x 18.0-20.5 μ with a mean of 22.5 x 18.75 μ and a length/width ratio of 1.1. Oocysts of *Eimeria* sp. "B" measured 22.5-28.10 x 16.85-20.60 μ with a mean of 26.25 x 19.75 μ and a length/width ratio of 1.3. The prepatent periods were 80-84 hours and 104-108 hours for *Eimeria* species "A" and "B" respectively. The minimum sporulation time at 30 C was between 4 and 8 hours for both species.

The incidence of infection was high in the adult (81%) and juvenal (79%) quail and lower in the immatures (40%). The intensity of infection was highest in the immature and juvenile quail. The adult birds apparently act as carriers of coccidia. There was no evidence of decimation of quail due to disease and/or other causes in the Okanagan Valley. There were no significant differences in the incidence of infection between the sexes or the four study areas. There were monthly variations in the incidence of infection, with the lowest incidence in June and the highest in August.

Six laboratory raised 6-week-old Japanese quail (*Coturnix coturnix*), eight 10-day-old Leghorn chicks (*Gallus domesticus*) and four 10-day-old laboratory raised California quail controls were inoculated *per os* with oocysts of *Eimeria* obtained from naturally infected California quail from the Okanagan Valley. Transmission attempts with the Japanese quail and the Leghorn chicks were unsuccessful. The California quail controls were successfully infected.

The sporulation process was studied, and was found to be similar to that described for other coccidia. The sporulation times and the percentage of oocysts sporulating at temperatures of 15,20,25,30 and 38 C indicate that the optimum sporulating temperature for both species is over 30 C and may be over 38 C.

This is the first report of *Eimeria* from California quail in British Columbia. Morphological characters of the oocysts indicate that the two species reported herein differ markedly from each other, as well as from the four chicken species, *Eimeria acervulina*, *E. mitis*, *E. tenella* and *E. maxima*, listed as occurring in California quail, and are apparently undescribed species. Records of the four chicken species listed above from California quail are considered erroneous.

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Adult California quail, male (front), female (behind).

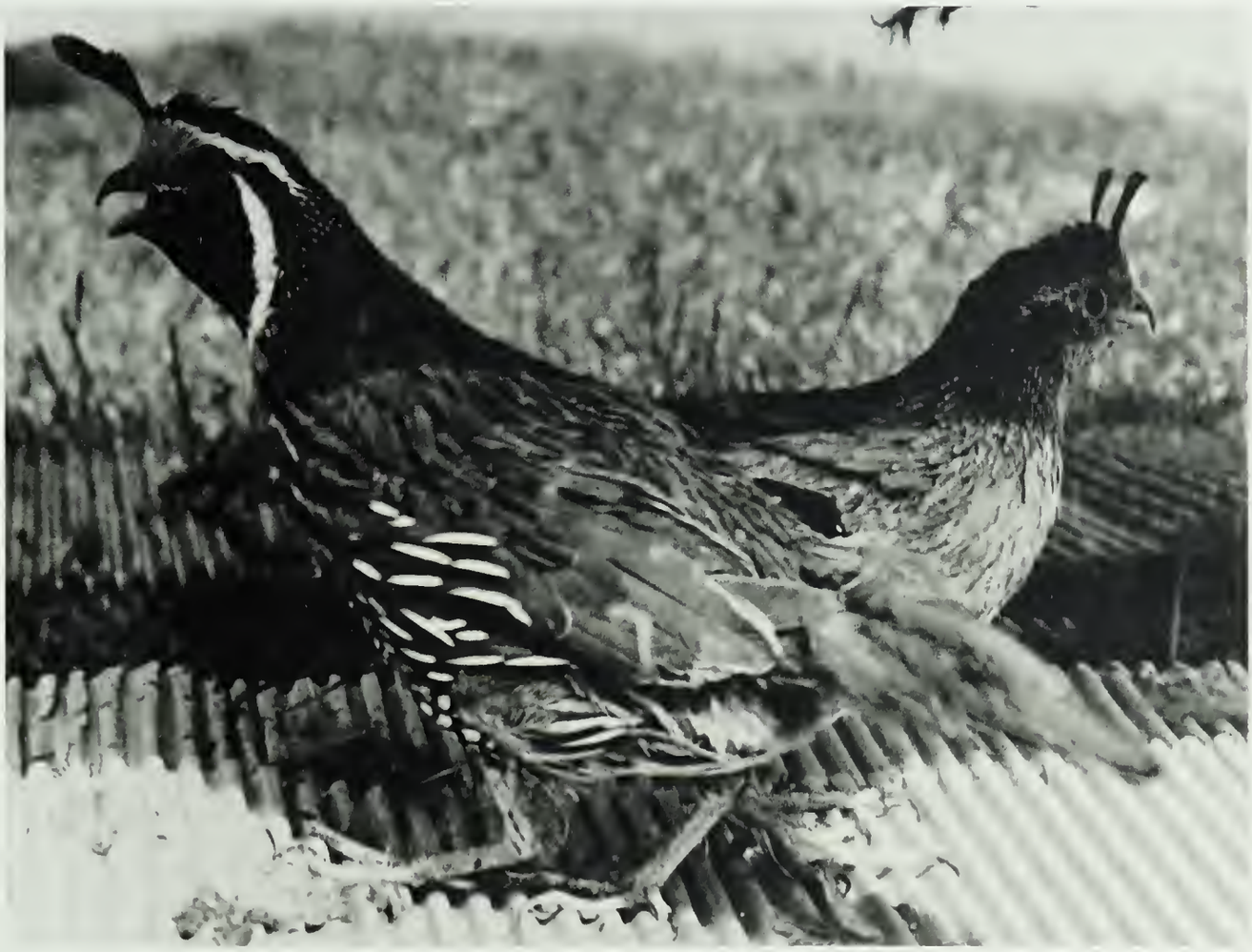


TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Eimeria in California quail

The coccidia of California quail (*Lophortyx californicus*) were unknown until the appearance of a monograph by Henry (3) on the species of coccidia in chickens and quail in California. She reported finding three species, *Eimeria acervulina, E. mitis and E. tenella,* in wild and captive California quail. She claimed to have transmitted all three species to domestic chickens, and to have infected chickens with turkey coccidia whose oocysts were identical with those of *E. acervulina* and *E. tenella*. Herman and co-workers (4-8) reported finding at least five species of *Eimeria* in wild and captive California quail, but attempts at transmitting four of the five species to domestic chickens were unsuccessful (4). These workers did not attempt to transmit the fifth species to domestic chickens, neither did they describe or name any of the five species found. Lewin (14) reported the presence of *Eimeria* in wild California quail, but like Herman and co-workers he neither described nor named them.

Levine and Becker (13) and Becker (1) listed, as occurring in California quail, the three species reported by Henry (3). Pellerdy (18) listed these three, plus *Eimeria maxima*, under California quail. Both Becker and Pellerdy placed question marks after the *Eimeria* species listed as occurring in California quail. Levine (12) discussed the reports of Henry (3) and Herman (4) and stated that despite Henry's claim to have infected chickens with turkey coccidia (whose oocysts were "identical" with those of *E. acervulina* and *E. tenella*), Tyzzer (24) was unable to infect the chicken, pheasant or quail with *E. meleagridis* from the turkey. Levine concluded that, in view of Herman's findings, it was rather unlikely that *E. tenella*, *E. acervulina* and *E. mitis* occur in California quail or bobwhite quail, and that the species which do occur in these hosts remained to be determined.

As a result of extensive work on the host specificity of *Eimeria* (9-12,20), and the negative results of the cross transmission studies by Herman and co-workers, it seems unlikely that the *Eimeria* species reported by Henry would occur in wild California quail. At present, then, *Eimeria* are known to occur in California quail but the species involved are unknown.

Pellerdy (19), on reviewing the work of other investigators, notably Henry (3) and Herman and co-workers (4-8), suggested that coccidiosis is one of the major infectious diseases of California quail. He goes on to say that the disease decimates the young individuals generation after generation. There is no evidence in the literature referred to by Pellerdy to suggest this. In addition, Chandler (2), in his work on California quail in the Okanagan Valley, B.C. (the majority of which birds were used for this study), found no evidence of any decimation.

California quail in the Okanagan Valley, B.C.

California quail were introduced into the Okanagan Valley, B.C.; the earliest record of colonization dates back to 1912 (15). At present, quail are well established and being non-migratory, are found in the area throughout the year. Their success at colonization is determined by aspects of climate, habitat and patterns of land use. The areas suitable for quail habitat are those which are brushy, receive less than fifteen inches of precipitation yearly, not more than two inches of precipitation in any winter month, and less than forty inches per year as snow. However, quail must have access to drinking water; therefore, they are associated with the irrigated areas of the Okanagan Valley, and are rarely found above the 2000 foot contour (15). This is due to the fact that, in this valley, irrigation is not practiced above the 2000 foot elevation.

The Okanagan Valley is a relatively broad U-shaped depression between the Columbia and Cascade mountain ranges in

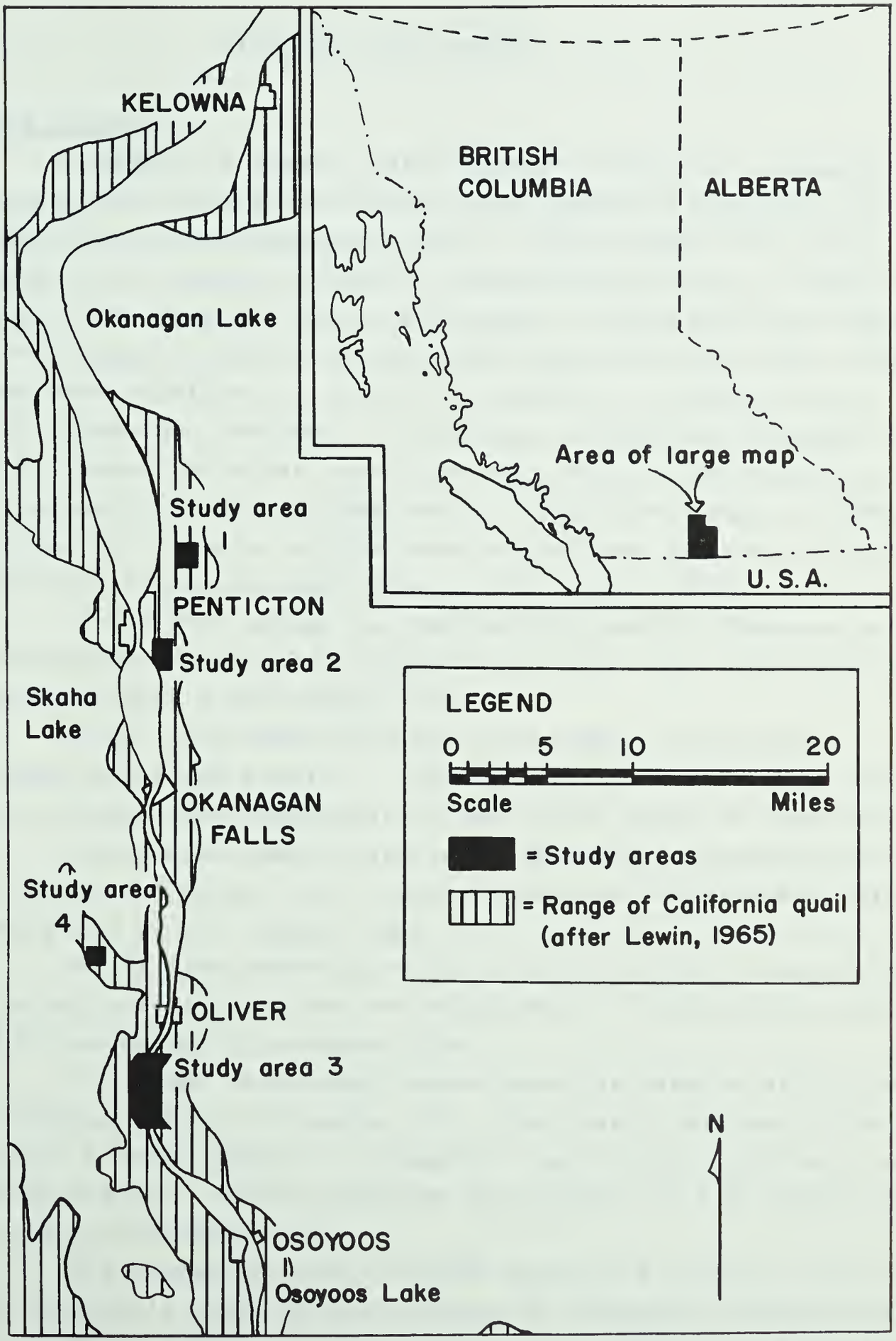
south central British Columbia. The valley extends from Salmon Arm south to the Canada-United States border. The approximate locations of the four study areas in the southern Okanagan Valley, from Naramata to just south of Oliver, are shown in Fig. 1. Of these areas, all but area 3 are orchards and are closed to public use.

My interest in the *Eimeria* of California quail was stimulated by discussions with Dr. V. Lewin, who had worked on the effects of *Eimeria* on the growth of the quail (14), and on the ecology of California quail in the Okanagan Valley (15). As Dr. Lewin and Mr. R.E. Chandler were initiating a study of the California quail of the Okanagan Valley, I took advantage of the opportunity and worked along with them.

Objectives

The objectives of this study were to find, on the basis of characters of the oocysts, sporogony, prepatent periods, and transmission studies, what species of *Eimeria* were present in California quail in the Okanagan Valley, B.C., and to investigate aspects of their ecology revealed by a general survey of the quail.

Figure 1. Map showing location of study areas and range of California quail in the southern Okanagan Valley. The range of California quail is based on Lewin (15). (Courtesy of R.E. Chandler).


KELOWNA
BRITISH COLUMBIA
ALBERTA
Okanagan Lake
Area of large map
Study area 1
PENTICTON
U. S. A.
Study area 2
Skaha Lake
LEGEND
0 5 10 20
Scale Miles
= Study areas
= Range of California quail (after Lewin, 1965)
OKANAGAN FALLS
Study area 4
OLIVER
Study area 3
N
OSOYOOS
Osoyoos Lake

MATERIALS AND METHODS

Field Studies

Samples of faeces, caecal and/or intestinal contents were obtained from 85 California quail captured from the four study areas in the Okanagan Valley. Field studies were conducted in the summer of 1965 in conjunction with Dr. V. Lewin and Mr. R.E. Chandler and in the summer of 1966 with Mr. Chandler. In 1965 samples from 62 of 106 birds captured in the four study areas were examined for oocysts of *Eimeria*. In 1966 a total of 23 birds was examined. In February of 1966 Mr. Chandler and I visited the study areas (February 15th to 19th) but we did not succeed in capturing any quail. During the summer of 1966 I joined Mr. Chandler at the study areas from June 6th to 13th, from July 18th to 24th and from August 14th to 18th.

Study area number one was located east of Okanagan Lake approximately four miles north of Penticton. The area contained mature orchards and bushy gullies.

Study area number two was also composed of mature orchards and bushy gullies. This area was located on the east side of Skaha Lake approximately four miles south of Penticton.

Study area number three was located approximately six miles south of Oliver on a levee adjacent to orchards and with brush piles at the levee's edge.

Study area number four was a mature orchard located two and a half miles north and two miles west of Oliver in an area mostly covered with ponderosa pine.

The first three study areas were the same as study areas one through three of Chandler (2). The fourth area was close to and in a habitat similar to Chandler's area four, but was in a different spot. A more extensive description of the study areas was given by Chandler (2).

The areas selected as study areas were chosen to facilitate Chandler's study of the effects of parasites and pesticides on California quail. In most instances we shared the same quail.

However, I did not have access to over forty quail collected by Dr. Lewin and Mr. Chandler during the earlier part of the study.

California quail were captured by shooting and trapping. Traps were constructed of one inch hexagonal mesh galvanized poultry netting and were baited with canary seed. The traps were kept in constant operation throughout the study periods and were examined twice daily, in the morning and in the evening. Trapped quail were removed from the traps, placed in jute bags and the traps rebaited. At the field laboratory the trapped quail were placed in individual, wire floored cages over paper to collect the faeces. These birds were usually kept for several hours, during which time their faeces were examined for oocysts of *Eimeria*. Some of the trapped birds that were infected were kept for a source of oocysts and were taken to the main laboratory at the Zoology Department, University of Alberta. The others were killed by thoracic compression and autopsied.

While checking the traps any untrapped quail seen were collected by shooting. Such birds were autopsied immediately upon returning to the field laboratory.

At autopsy, the quail were classified as adults, immatures or juveniles (14). Juvenal quail were aged, to weeks of age, by primary feather replacement (21). Adult birds were sexed by their plumage while the juvenal birds were sexed by examination of their gonads. Samples of the contents were squeezed from the caeca and intestines and examined for oocysts. Samples containing oocysts were mixed with 2.5% potassium dichromate solution, placed in petri dishes to a depth of 1 cm or less and allowed to sporulate at room temperature. In this way the faeces, caecal and intestinal contents of all live birds were examined. Only the caecal and intestinal contents of shot birds were examined.

Faecal, caecal and/or intestinal samples collected by Dr. Lewin and R. Chandler in 1965 or by Mr. Chandler in 1966

were placed in phials containing 2.5% potassium dichromate solution and either sent or brought to the main laboratory. Each phial contained a sample from one quail and was labeled with the autopsy number of the bird. Pertinent data regarding the quail were then obtained from the autopsy records of Mr. Chandler. At the main laboratory the entire sample was placed in a 15 ml. centrifuge tube and centrifuged at about 2,000 revolutions per minute for six minutes. The supernatant was then decanted and the contents examined for oocysts.

Examination for the presence of oocysts was by direct smear and by flotation in concentrated sodium chloride solution. As soon as a bird was found to be infected with *Eimeria*, the intensity of infection (oocysts/gram) was measured by the Stoll dilution egg count method (23). This method was used because of its convenience and simplicity of use in the field.

Unsporulated and sporulated oocysts were examined for details of morphology with a 100 X oil immersion achromatic objective. Measurements were made under oil using an ocular micrometer, converting the final figures to microns. All measurements of unsporulated oocysts were made on fresh samples. Measurements of sporocysts from sporulated oocysts were made after being liberated from the oocysts by gentle pressure on the coverslip against the microscope slide (Fig. 7). Photomicrographs were made using a 100 X oil immersion objective and a Pentax-Spotmatic 35 mm camera.

Transmission Studies

Laboratory-raised Japanese quail (*Coturnix coturnix*), obtained from Dr. J. Lauber, Leghorn chicks (*Gallus domesticus*), purchased from a local hatchery, and California quail, incubated from eggs purchased from two game farms in California, were maintained separately in wire floored cages. The birds were fed non-medicated chick starter crumbles, and faeces were collected on paper below the wire floors. Daily faecal samples were examined for oocysts to see that no natural infections had occurred.

One lot of oocysts, obtained from naturally infected California quail from the Okanagan Valley and consisting of a mixture of species "A" and "B", which had been sporulated in 2.5% potassium dichromate solution and stored at 4 C, was used in the transmission studies. The oocysts were washed free of potassium dichromate by repeated centrifugation, suspended in distilled water and the number in the suspension calibrated by the McMaster technique (25).

Three eight-week-old Japanese quail were inoculated *per os* with approximately 2,000 oocysts per bird, three others with 5,000 oocysts per bird, and three were kept as uninoculated controls. Four ten-day-old Leghorn chicks were inoculated with 1,000 oocysts per bird, four others with 5,000 oocysts per bird, and four were kept as uninoculated controls. Two ten-day-old California quail were inoculated with 500 oocysts per bird, two others with 1,000 oocysts per bird, and two were uninoculated. The infections in the California quail were deliberately made light so as to avoid death from coccidiosis.

Following inoculation, faecal samples were collected on moist paper placed below the wire floors. The total faeces of each group of Japanese quail and Leghorn chicks were examined every 24 hours by the direct centrifugal coverslip flotation technique (11); fresh faeces of the California quail were examined every 12 hours. At the end of 14 days one bird from each group of Japanese quail or Leghorn chicks was killed and the entire intestinal and caecal contents removed and examined for oocysts as outlined above. The remaining birds in these groups were observed for an additional seven day period, with daily faecal examination, after which time they were killed and the total intestinal and caecal contents examined by Sheather sugar flotation (11).

Oocysts which were shed by the infected California quail at different periods after inoculation were isolated, sporulated in 2.5% potassium dichromate solution and stored in phials at 4 C. These oocysts were later identified as

species "A" or species "B" and used for the study of prepatent periods.

Prepatent Periods

The prepatent periods for the two species found were determined by inoculating parasite-free California quail chicks with single-species inocula from the transmission studies. Each bird was inoculated *per os* with approximately 1,000 oocysts. Fresh faecal samples were collected on moist paper every 12 hours for the first 3 days and every 4 hours thereafter. These samples were examined by coverslip flotation. The oocysts obtained from these experiments were sporulated in 2.5% potassium dichromate solution and stored at 4 C, or were used for studies of sporulation times or the sporulation process itself.

Sporulation

The sporulation time was determined at 5 different temperatures, 15,20,25,30 and 38 C, done at different times and with different lots of fresh oocysts. Each experiment was duplicated; the final results are the averages of the two experiments. Sporulation times were determined by mixing fresh faecal samples (within five minutes of defecation) from infected California quail with 2.5% potassium dichromate solution, pouring it into 9 cm petri dishes to a depth of 1 cm or less, incubating in a Fisher low temperature incubator and examining at 2, 4 and 8 hour intervals. The stage of sporulation at each temperature and at each examination period was ascertained by examining 100 oocysts and determining whether they were degenerated (Dg) or contained sporonts (Sr), sporoblasts (Sb), sporocysts (Sc) or sporocysts with sporozoites (Sz). The experiments at 30 and 38 C were terminated when all 100 oocysts were fully sporulated or degenerated. In the other temperature ranges (15, 20 and 25 C) the experiments were followed through 48 hours to determine the percent sporulated at 48 hours.

The sporogony of *Eimeria* species "B" was studied in hanging drop preparations at room temperature.

RESULTS

Field Survey

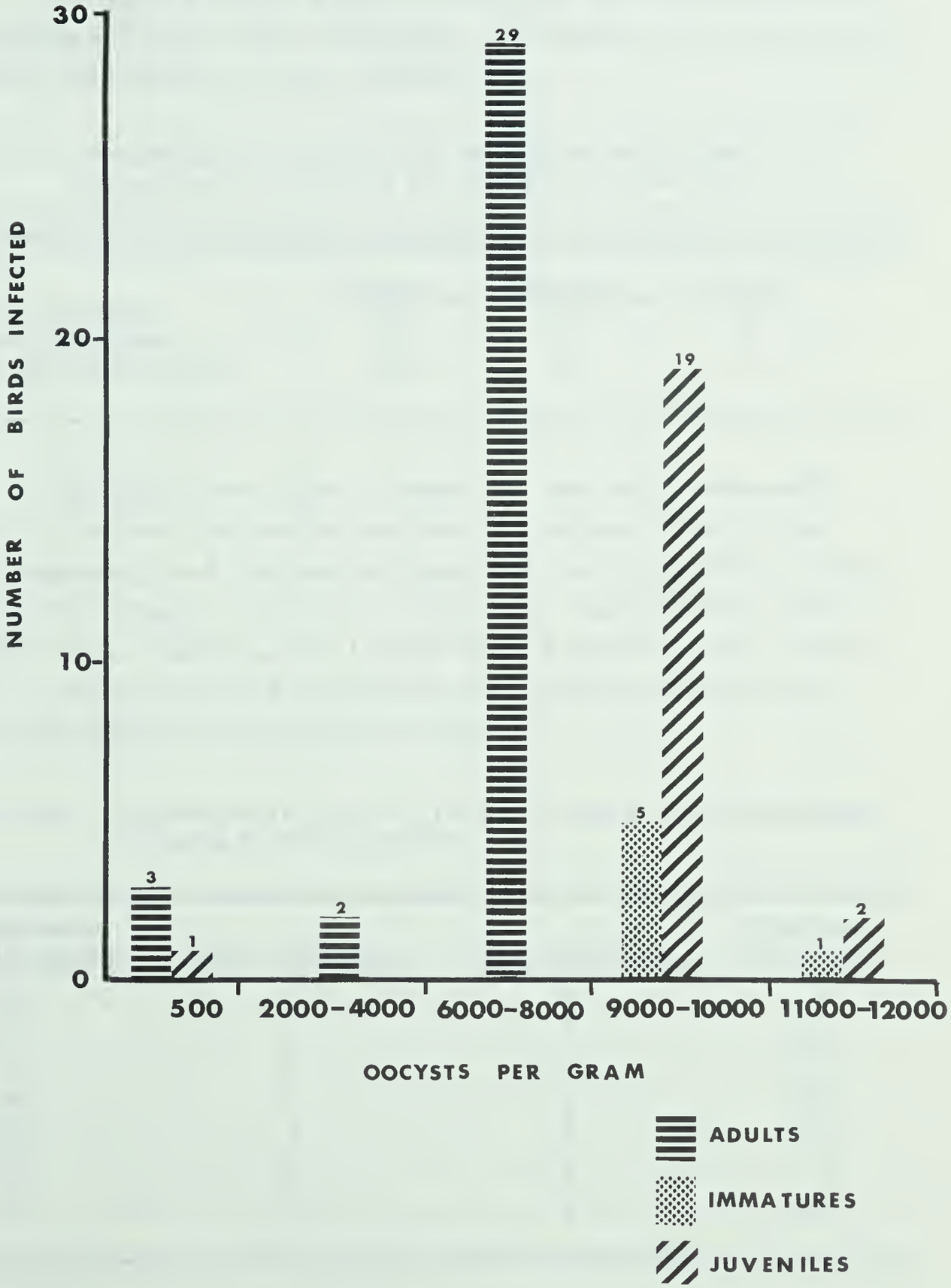
Examination of faecal, caecal and/or intestinal samples revealed a high incidence of coccidian infection in wild California quail in the Okanagan Valley, B.C. Seventy three per cent of the 85 quail examined were infected (Table I). No clinical manifestations of coccidiosis were observed. The results suggest that subclinical infections represent the normal course of events in the Okanagan Valley.

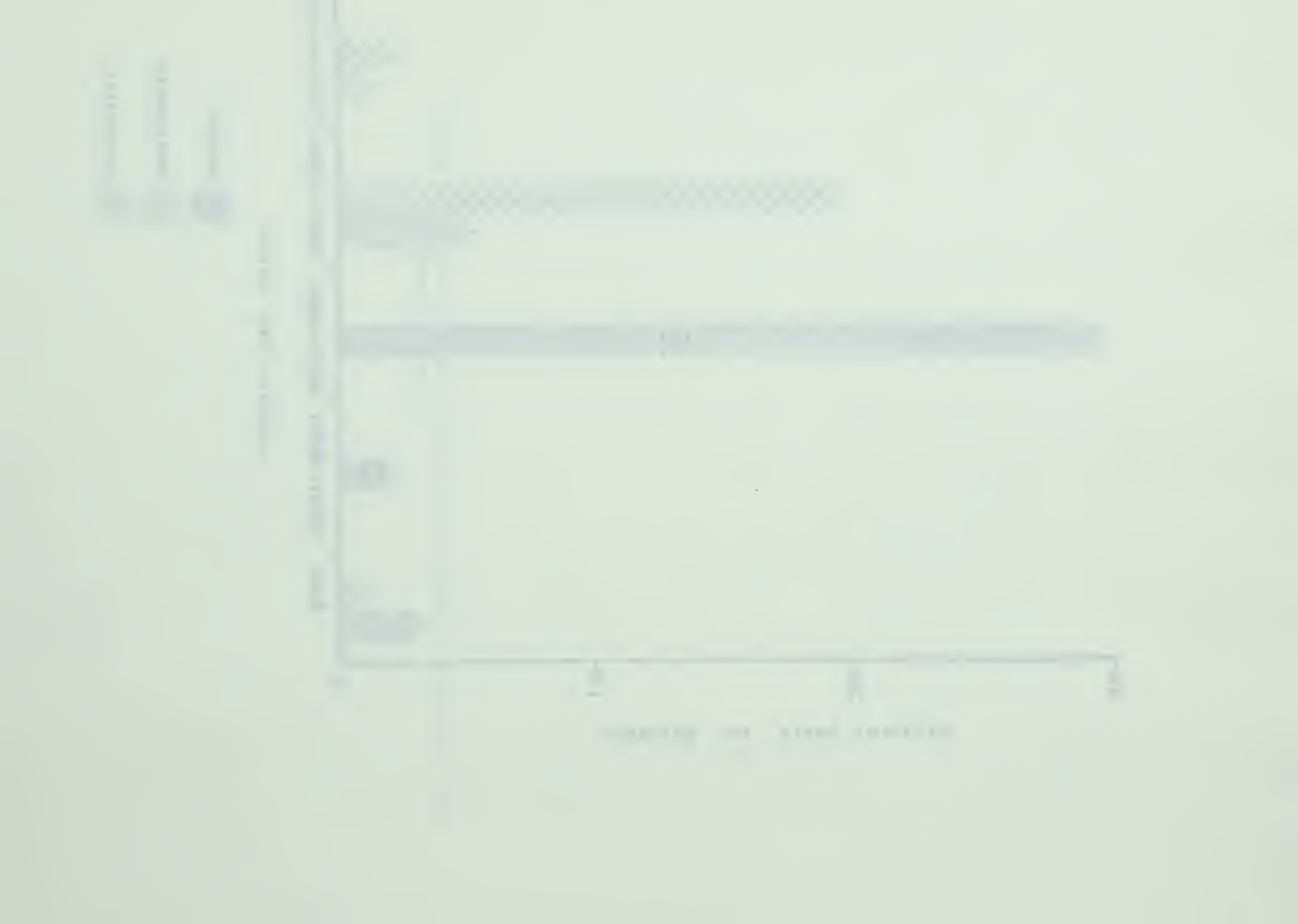
Table I. Extensity and intensity of *Eimeria* species in California quail from the Okanagan Valley.

	Adults	Immatures	Juveniles	Total
Number examined	42	15	28	85
Number positive	34	6	22	62
Extensity (per cent)	81	40	79	73
Intensity (range: oocysts/gm)	500-9000	500-12000	500-12000	

Incidence of infection as indicated by the presence of oocysts was high in the adults and juveniles and lower in the immatures, 81, 79 and 40 per cent respectively (Table I). A Chi-square test showed this variation to be significant ($X^2=10.40$, $p>.01$). Examinations of samples from birds frequently revealed a much higher intensity of infection (oocysts/gram) in some birds than in others (Fig. 2). The juveniles and immatures had higher intensities of infection than the adults.

Figure 2. Intensity of infection in adult, immature and juvenile quail.


30
20
10
0
NUMBER OF BIRDS INFECTED
3
1
2
29
5
19
1
2
500
2000-4000
6000-8000
9000-10000
11000-12000
OOCYSTS PER GRAM
ADULTS
IMMATURES
JUVENILES



The males had a higher incidence of infection than the females (Table II), although a Chi-square test indicated that the difference is not significant.

Table II. Incidence of infection of California quail in relation to the sex of the birds examined.

	Males	Females	Total
Number examined	40	45	85
Number infected	32	30	62
Incidence (per cent)	80	67	73

No quail less than 6 weeks of age were examined. Table III shows the number and age of juvenal quail that were examined, and the percentage that were infected at each age level. Higher levels of infections were found at the earliest age, while lower levels were found in older juvenal quail. One 7-week-old juvenal quail had an intensity of infection of 500 oocysts/gram (Fig. 2).

Table III. Approximate age of juvenal quail and percentage infected with *Eimeria*.

Approximate age in weeks	No. examined	No. infected	Percent infected
1-5	0	0	0
6	4	3	75
7	3	3	100
8	3	2	67
9	2	2	100
10	3	3	100
11	0	0	0
12	2	0	0
13	2	1	50

No significant differences of incidence of infection between study areas were apparent (Table IV).

Table IV. Incidence of infection of quail with *Eimeria* spp. in relation to site of capture.

	Adults	Immatures	Juveniles	Overall
Area 1				
Number examined	14	7	13	34
Number infected	13	3	10	26
Per cent infected	93	43	77	76
Area 2				
Number examined	9	1	3	13
Number infected	7	1	2	10
Per cent infected	78	100	67	77
Area 3				
Number examined	11	6	10	27
Number infected	8	2	8	18
Per cent infected	73	33	80	67
Area 4				
Number examined	8	1	2	11
Number infected	6	0	2	8
Per cent infected	75	0	100	73

The data in Table V suggest some monthly differences in incidence of infection. The highest number of birds examined and the highest incidence of infection were obtained in August of both years, while the lowest number of birds examined and the lowest incidence of infection were found in June of both years. The percentage of infected birds was approximately the same in both years, 74% for 1965 and 70% for 1966, hence the data for both years was combined. Although the number of birds examined was small, a Chi-square test indicated that the variation was significant ($X^2=9.46$; $.05>p>.01$).

Table V. Monthly variation in number of birds captured and incidence of infection.

	June	July	August	September	Total
1965					
Number examined	2	15	39	6	62
Number infected	1	9	32	4	46
Per cent infected	50	60	82	67	74
1966					
Number examined	3	8	8	4	23
Number infected	0	6	7	3	16
Per cent infected	0	75	88	75	70
Total for both years					
Number examined	5	23	47	10	85
Number infected	1	15	39	7	62
Per cent infected	20	65	83	70	73

Transmission Studies

No development of *Eimeria* from naturally infected California quail was obtained in the six laboratory raised Japanese quail or the eight laboratory raised Leghorn chicks that were inoculated. The four laboratory raised California quail controls were all successfully infected. The uninoculated California quail did not become infected. California quail that were inoculated at other times were also successfully infected. Infected birds showed ruffled feathers, loss of appetite and droopyness, symptomatic of mild disease, but no bloody stools or other evidences of more severe coccidiosis were noted.

Prepatent Periods

The first oocysts of species "A" were detected by sugar flotation at 80 hours after inoculation and a shower of oocysts started 84 hours after inoculation. The first oocysts of species "B" were detected by sugar flotation 104 hours after inoculation and a shower of oocysts was detected 108 hours after inoculation.

The Sporulation Process

Table VI shows the mean percentage of *Eimeria* species "B" at each stage of the sporulation process after various times at 30 and 38 C. Values given are averages of data from two separate experiments. The minimum sporulation time of species "B" was between four and eight hours at 30 C.

Table VI. Mean percentage of *Eimeria* species "B" oocysts at each stage of sporulation for 2 temperatures.

	Percent of Oocysts*									
	30 C					38 C				
Hours of sporulation	Dg	Sr	Sb	Sc	Sz	Dg	Sr	Sb	Sc	Sz
0	-	100	-	-	-	-	100	-	-	-
4	3	-	8	89	-	6	-	4	90	-
8	3	-	-	5	92	7	-	-	-	93
12	6	-	-	-	94					

*Dg=degenerated oocyst; Sr=sporont; Sb=sporoblast; Sc=sporocyst; Sz=sporozoite.

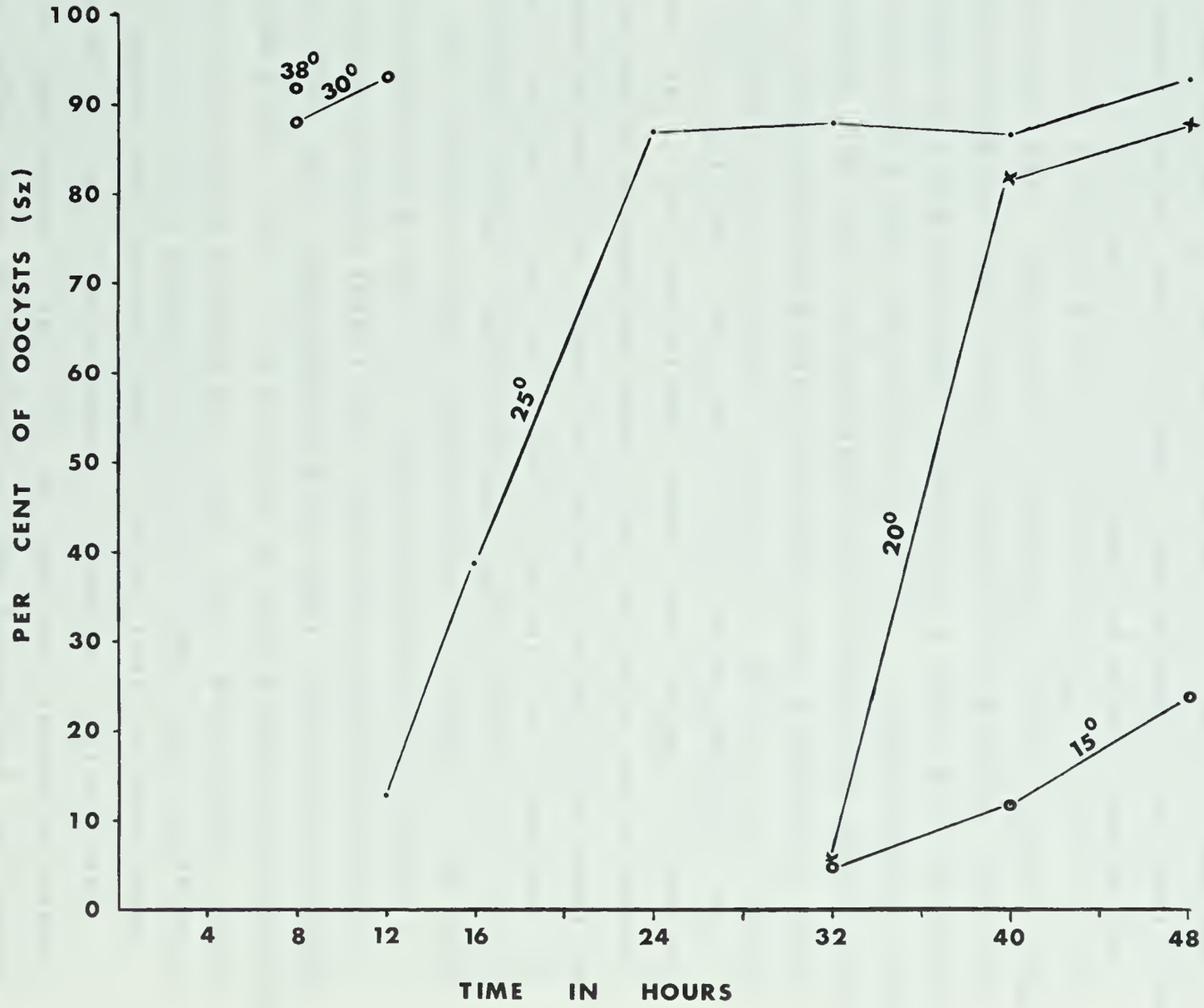
Table VII summarizes the mean percentage of *Eimeria* species "A" at each stage of the sporulation process after various times at 15,20,25,30 and 38 C. Values given are averages of data from two separate experiments. Sporulation was very rapid, and was accelerated by an increase in temperature (Fig. 3). The rapid completion of sporogony (the sporulation process) at 30 and 38 C suggests an optimum sporulation temperature of at least 30-38 C, perhaps higher. The minimum sporulation time of species "A" was between four and eight hours at 30 C.

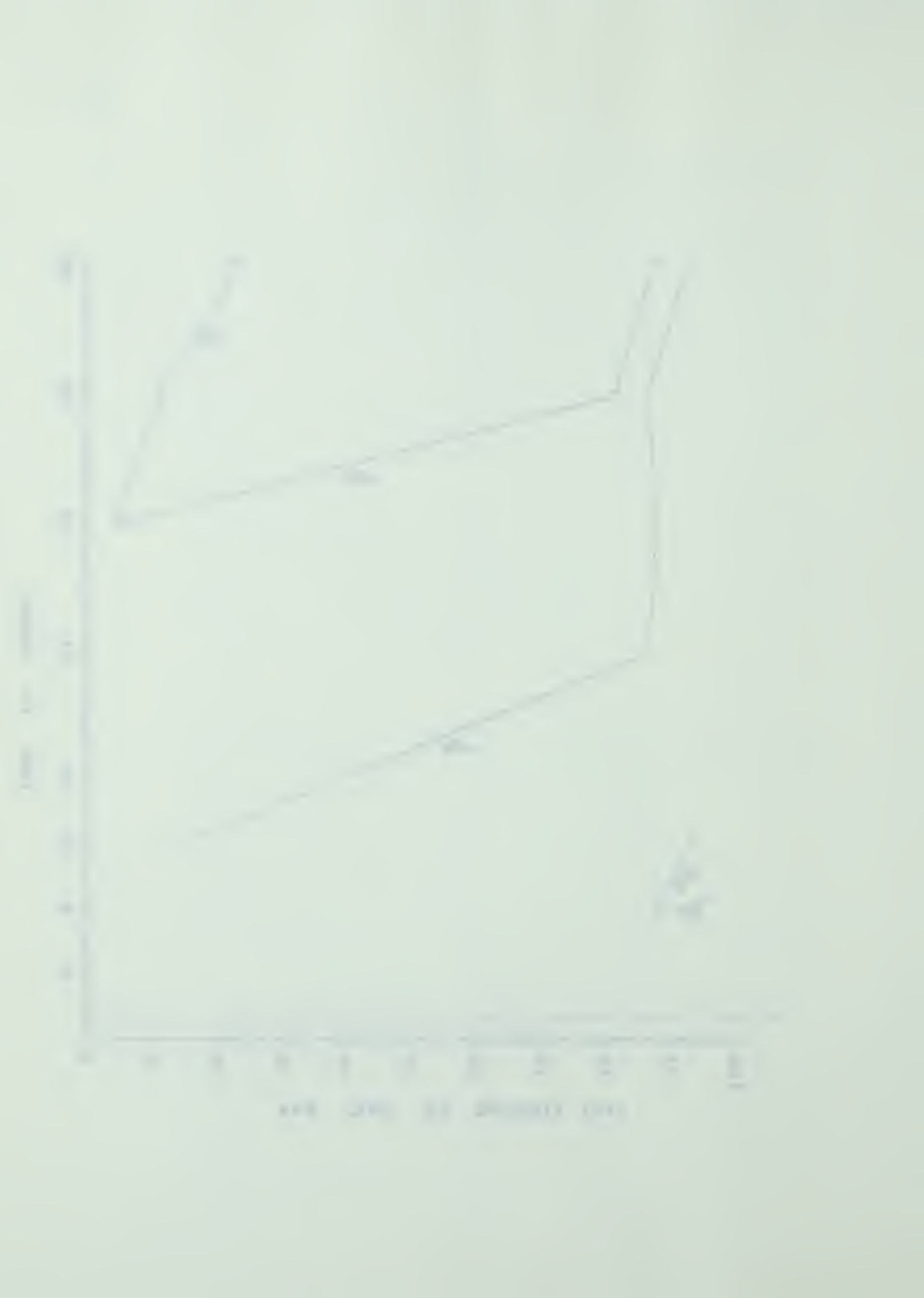
Table VII. Mean percentage of *Eimeria* species "A" oocysts at each stage of sporulation for five temperatures of incubation.

Hours of sporulation	Percent of Oocysts*																									
	15 C					20 C					25 C					30 C					38 C					
	Dg	Sr	Sb	Sc	Sz	Dg	Sr	Sb	Sc	Sz	Dg	Sr	Sb	Sc	Sz	Dg	Sr	Sb	Sc	Sz	Dg	Sr	Sb	Sc	Sz	
0	-	100	-	-	-	-	100	-	-	-	-	100	-	-	-	-	100	-	-	-	-	100	-	-	-	
4	1	-	99	-	-	6	-	94	-	-	3	2	93	2	-	2	-	7	91	-	7	-	6	87	-	
8	6	-	94	-	-	5	-	85	10	-	4	-	87	9	-	2	-	2	8	88	8	-	-	-	92	
12	9	-	91	-	-	3	-	83	14	-	7	-	2	78	13	4	-	-	3	93						
16	8	-	80	12	-	9	-	80	11	-	10	-	1	50	39											
24	6	-	54	40	-	4	-	80	16	-	3	-	-	10	87											
32	10	-	15	70	5	1	-	13	80	6	2	-	1	9	88											
40	5	-	10	73	12	6	-	-	12	82	3	-	-	10	87											
48	8	-	-	68	24	5	-	-	7	88	5	-	-	2	93											

*Dg=degenerated oocyst; Sr=sporont; Sb=sporoblast; Sc=sporocyst; Sz=sporozoite.

Figure 3. Mean percentage of fully sporulated *Eimeria* sp. "A" oocysts at various temperatures.


PER CENT OF OOCYSTS (Sz)
100
90
80
70
60
50
40
30
20
10
0
38°
30°
25°
20°
15°
4
8
12
16
24
32
40
48
TIME IN HOURS



The sporulation process (Fig. 4) was followed in *Eimeria* species "B" in hanging drop preparations at room temperature. The fresh unsporulated oocyst had coarse granular sporoplasm occupying its central area (Fig. 4a). A nucleus was present but not well defined. Approximately one hour after the start of sporogony the sporoplasm had moved towards the walls of the oocyst and the nucleus showed up clearly in its centre (Fig. 4b). This was the beginning of the sporont stage. The next stage was that of the nuclear streak where there was a halving of the sporoplasmic mass with a clear area extending from end to end down the centre (Fig. 4c). Approximately three hours after the start of sporogony the nucleus was again poorly defined, the sporoplasm was pear shaped and a streak of protoplasm seemed to be attached to one of the poles of the oocyst (Fig. 4d). This was the start of the "Buckel-stadium" of Metzner (16). The protoplasmic mass then became ellipsoidal and the entire sporoplasm moved from the poles and occupied a crosswise position within the oocyst, forming the completed "Buckel-stadium" (Fig. 4e). Shortly after the formation of the "Buckel-stadium" the sporoplasm again formed a continuous mass. The nucleus became prominent and cleavage of the sporoplasm started (Fig. 4f). At the next observation period (five hours of incubation) four ball-like sporoblasts had formed (Fig. 4g). Approximately one hour later four pyramids had formed (Fig. 4h). The nucleus in each pyramid was undefined and one end of the pyramid was clear for a short while, then became filled with granular material. After the formation of the pyramids they became constricted at their pointed ends and developed into four separate, irregular, ball-shaped granular sporocysts (Fig. 4i). At the next period of observation (seven hours of incubation) the sporocysts had become ellipsoidal, very granular, and had a Stieda body at one end of the sporocyst (Fig. 4j). Between 13 and 14 hours after the beginning of sporogony the oocyst was fully sporulated, containing four ellipsoidal, well defined sporocysts, each with two sporozoites and a granular residuum (Fig. 4k).

Figure 4 (a,b,c,d,e,f,g,h,i,j,k). Photomicrographs of the sporulation process in oocysts of *Eimeria* species "B".

a. Oocyst freshly passed in faeces.

b. Oocyst with sporont showing nucleus.

c. Oocyst showing the nuclear streak stage.

d. Oocyst showing a protoplasmic streak. This is the start of the "Buckel-stadium."

e. Oocyst showing a square sporont or the "Buckel-stadium."

f. Oocyst with sporont beginning cleavage.

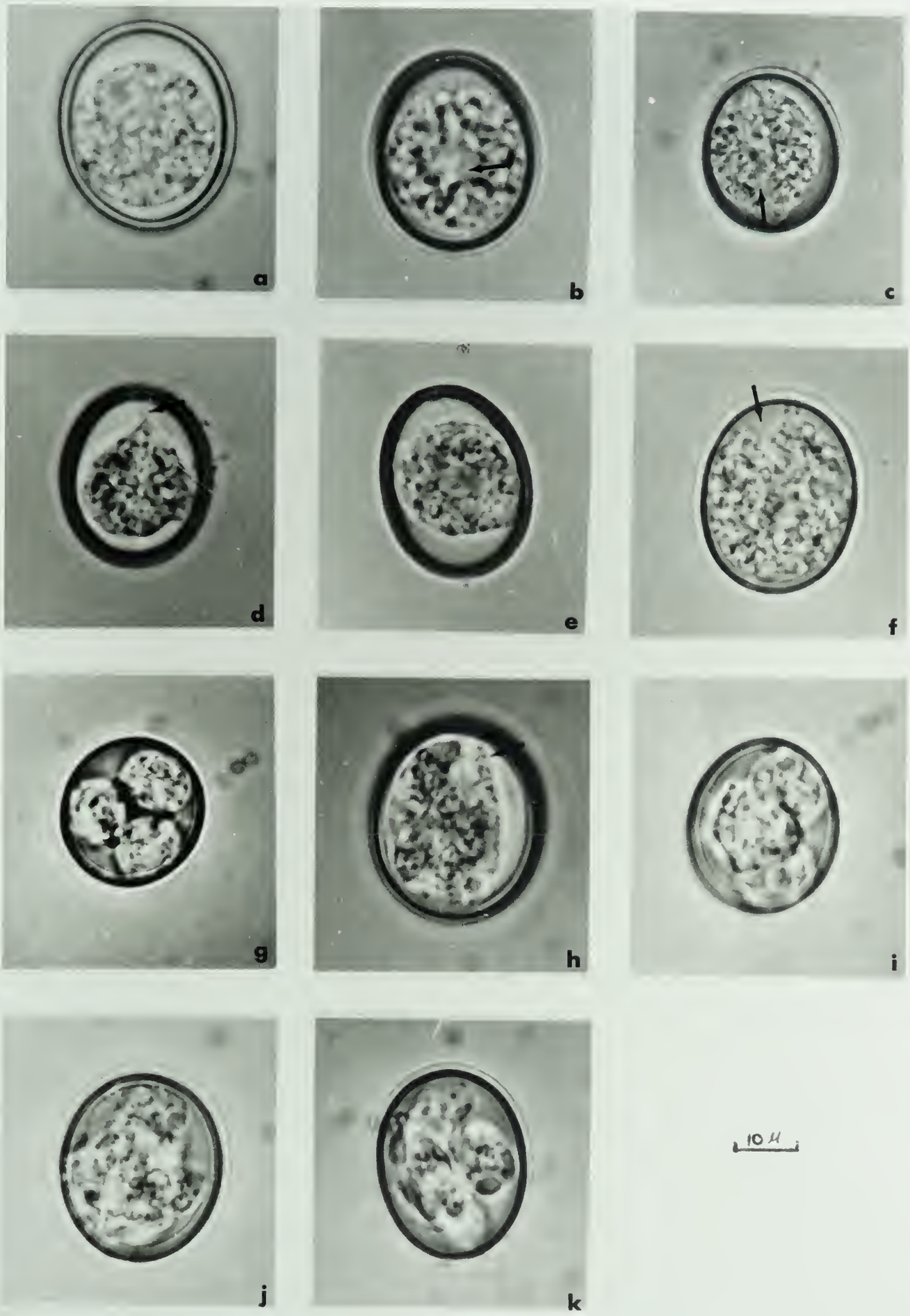
g. Oocyst showing four ball-shaped sporoblasts.

h. The pyramid stage showing four pyramids.

i. Oocyst with four newly forming ball-shaped sporocysts.

j. Oocyst with granules in the sporocyst.

k. Sporulated oocyst with four sporocysts, each containing two sporozoites and granular residuum.


a
b
c
d
e
f
g
h
i
j
k
10 µ

Sporogony in species "A" was similar; the process in both agreed with those described by Metzner (16), Nieschulz (17) and Reich (22). Due to the length of the process and the brevity of some stages, only the more salient stages of development have been recorded.

Characters of species of *Eimeria* from California quail

Table VIII summarizes the characters of the two species of *Eimeria* found in California quail from the Okanagan Valley, and compares them with the characters of the three species of *Eimeria* of chickens reported from California quail, but not described, by Henry (3) and *E. maxima*, listed by Pellerdy (18) from California quail.

All measurements are given in microns; values in parentheses are means.

Eimeria species "A" (Fig. 5)

Description: Oocysts subspherical; sporoplasm fills the entire oocyst; oocyst wall smooth, colourless, two layered, thick; micropyle absent; 75 oocysts measured 20.6-26.3 x 18.0-20.5 (22.5 x 18.75); length/width ratio 1.1; oocysts with four sporocysts, polar granule present, residuum absent. Sporocyst broadly ovoid; 75 sporocysts measured 12.75-14.25 x 6.0-8.25 (13.75 x 7.5); length/width ratio 1.7 to 2.12; prominent Stieda body. Sporozoites bean-shaped, lying lengthwise in the sporocyst, with two conspicuous refractile globules. Minimum sporulation time between 4 and 8 hours at 30 C. Prepatent period 80 to 84 hours.

Host: *Lophortyx californicus* (California quail), wild.

Geographic location: Okanagan Valley, British Columbia.

Location: Intestines and caeca.

Remarks: The oocysts described do not resemble those of any of the species reported from California quail by Henry (3) or

listed by Pellerdy (18). Species "A" is distinguished from species "B" by its short, broadly ovoid sporocysts; its absence of a sporocyst residuum; its shorter prepatent period; its subspherical rather than ovoid oocyst; and the sporoplasm which fills the entire oocyst.

Eimeria species "B" (Fig. 6)

Description: Oocyst ovoid, sporoplasm distinctly greenish and granular; sporoplasm forms a ball in the centre of the oocyst; oocyst wall smooth, colourless, two layered, thick; micropyle absent; 75 oocysts measured 22.50-28.10 x 16.85-20.6 (26.25 x 19.75); length/width ratio 1.3 to 1.4; oocysts with four sporocysts, polar granule present, residuum absent. Sporocyst ellipsoidal, pointed at one end; 75 sporocysts measured 13.1-15.0 x 5.6-8.4 (14.0 x 6.5); length/width ratio 2.3; prominent Stieda body. Sporozoites sausage-shaped, located near the pointed end, with two conspicuous refractile globules. Minimum sporulation time between 4 and 8 hours at 30 C. Prepatent period 104 to 108 hours.

Host: *Lophortyx californicus* (California quail), wild.

Geographic location: Okanagan Valley, British Columbia.

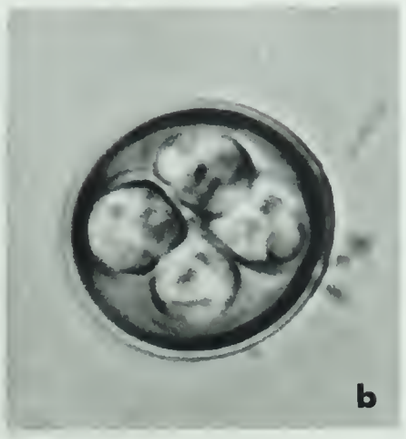
Location: Intestines and caeca.

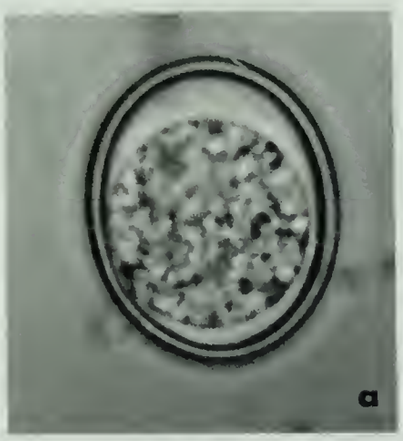
Remarks: The oocysts described resemble the photograph of *Eimeria tenella* from California quail published by Henry (3), but not those of *E. tenella* of other authors. Species "B" is distinguished from species "A" by its ovoid shaped oocyst, its larger oocysts and sporocysts (although their lengths overlap, both the oocysts and sporocysts of species "B" are larger and have bigger length/width ratios); its abundant granular sporocyst residuum; its longer prepatent period; its shorter sporulation period; and the sporoplasm, which does not fill the entire unsporulated oocyst.

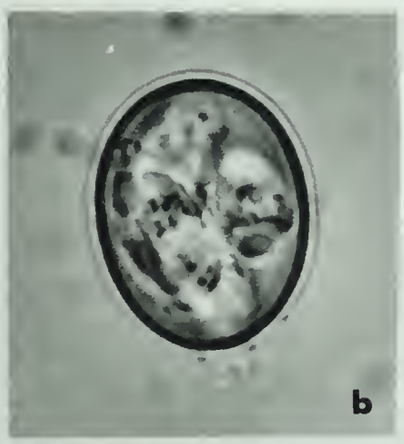
Figure 5. *Eimeria* species "A", (a) unsporulated oocyst, (b) sporulated oocyst.

Figure 6. *Eimeria* species "B", (a) unsporulated oocyst, (b) sporulated oocyst.

Figure 7. *Eimeria* species "B" showing 3 sporocysts outside the oocyst wall and 1 inside.







10 µ

Table VIII. Characters of species of *Eimeria* from chickens and quail.

			Oocyst characters*								Sporocyst* characters			
			Size (μ)											
Species	Hosts	Locality	Range Length	Range Width	Mean Length	Mean Width	Colour	Shape	Surface	Micropyle	Polar gran.	Residuum	Residuum	Stieda body	Min. spor. hrs.	Pre. pat.period-hrs
E.acervulina (Tyzzer)	Chicken Calif. quail	World-wide	17.7-20.2	13.7-16.3	18	14.6	Colourless	Ovoid	Smooth	+ ?	+	-	-	+	17	97
E.maxima (Tyzzer)	" "	"	21.5-42.5	16.5-29.8	30.5	20.7	Yellowish	Ovoid	Rough or Smooth	-	+	-	-	+	30	123
E.mitis (Tyzzer)	" "	"	14.3-19.6	13.0-17.0	16.2	16.0	"	Subspherical	Smooth	-	+	+	-	+	18	99
E.tenella (Railliet & Lucet)	" "	"	19.5-26.0	16.0-22.8	22.0	19.0	Colourless	Ovoid	"	-	+	+	-	+	18	138
**E.coturnicis (Chakravarty & Kar)	Japanese quail	India	26.4-38.8	19.8-26.4				Oval		-		-	+			
Eimeria sp."A"	Calif. quail	Okanagan	20.5-26.3	18.0-20.5	22.5	18.75	Colourless	Subspherical	Smooth	-	+	-	-	+	4-8†	80-84
Eimeria sp."B"	"	Valley	22.5-28.1	16.85-20.6	26.25	19.75	"	Ovoid	"	-	+	-	+	+	4-8†	104-108

* Measurements, minimum sporulation times, and prepatent periods for *E. acervulina*, *E. maxima*, *E. mitis* and *E. tenella* modified after Levine (11).
**Characters of *E. coturnicis* after Pellerdy (19).
† Minimum sporulation time at 30 C.

DISCUSSION

The present study is the first on the coccidia of California quail in British Columbia. Coccidia were found, at high incidences of infection, in California quail from all four study areas in the Okanagan Valley. Since these study areas encompassed all of the habitats frequented by California quail in the southern Okanagan Valley, it is probable that these coccidia are widely distributed throughout this geographical area.

The incidence of infection is high in the adult and juvenal birds but lower in the immatures, due perhaps, to their social behaviour and feeding habits, through which the adults, which are carriers of coccidia, are in closer contact with the juveniles than with the immature birds. Perhaps this accounts for the high infection rate in the youngest juvenal birds examined; this infection rate is lower in the older juvenal quail.

Herman et al. (7,8) showed considerable variation in prevalence of coccidia in wild California quail at different seasons of the year. Although quail were collected only during the summer months in the present study, and although the sample of birds examined was small, the data suggest seasonal variations in incidence of infection which differ from those shown by Herman et al. These workers (7,8) found the highest incidence in April, decreasing thereafter to a low in September. I found that the incidence of infection in the southern Okanagan Valley was highest in August and September and lowest in June. These differences probably reflect differences in the general climate of California and interior British Columbia. The variation I found could be due to the relative absence of available sporulated oocysts in the spring, resulting from the cold weather of winter and early spring.

Herman (4) reported that four of the five species he found and studied extensively caused losses in young quail

raised at state-owned game farms in California. He also states that field census studies indicate that there is a large unexplained loss in the wild quail under twelve weeks old. However, no data were available on the significance of, or probable losses due to coccidia in the wild. Lewin (14) reported no pathological condition of any part of the gastro-intestinal tract in over five hundred quail he autopsied, despite a high incidence of coccidia. There is no evidence in the above statements to warrant the statement made by Pellerdy (19) that "coccidiosis is one of the major infectious diseases of the California quail, and decimates the young individuals generation after generation." From personal observations, and from discussions with R. Chandler, there is no evidence that wild California quail in the Okanagan Valley are "decimated" either by disease or by any other factors. The results of my study suggest that subclinical infection, coccidiasis, represents a more normal course of events than does acute infection, coccidiosis.

The studies on sporogony indicated that an increase in the incubation temperature (within the temperatures used) accelerated the process and lessened the sporulation time. The optimum temperature for sporogony in these species is between 30 and 38 C, or possibly higher, well above the optimum sporulation temperatures for most species of *Eimeria* from Galliformes (11). Temperatures of 40 and 41 C have been recorded as being lethal to oocysts of some species of *Eimeria* (19). This concurs with what Dr. J. Mahrt (personal communication) found in his studies with *Isospora rivolta* (Protozoa: Eimeriidae), of the dog. At the other end, 15 C seems to approach the lower limits at which sporulation can occur, since at this temperature only about one quarter of the oocysts sporulated in a 48 hour observation period compared to 88% and 93% at 20 and 25 C respectively.

Sporogony is similar in morphological detail to that of *Coccidium cuniculi* (=*E. stiedae*) (16), of *Eimeria pfeifferi* (17),

and of *Eimeria stiedae* (22). Dr. J.L. Mahrt (personal communication) also found similar stages in his study on the sporogony of *Isospora rivolta*. The only differences in sporogony of these species were differences in the time necessary for stage to stage development. Apparently, the sporulation time is characteristic of each species. Like Metzner (16), I found that the accumulation of granules in the cytoplasm renders the observation of cytological processes very difficult during certain stages of sporogony. Owing to the brevity of certain developmental stages it is difficult to record every stage of sporogony.

Research on the *Eimeria* of chickens (9,10,20), and the *Eimeria* in general (11,19) has indicated that they are a group of highly stenoxenic parasites. The results of the transmission experiments in this study, and the unsuccessful attempts of Herman and co-workers (4-8) at infecting domestic chickens with quail coccidia, seem to indicate that the coccidia of quail are also relatively stenoxenic. These results force one to think that Henry's reports on finding and successfully transmitting coccidia from quail to domestic chickens, and also transmitting turkey coccidia to domestic chickens were inaccurate. In addition, the photograph she published as "*E. tenella*" from California quail (3) does not resemble the *E. tenella* of other authors, but does resemble the species listed as *Eimeria* sp. "B". Among the similarities are the shape of the oocyst, the position of the rounded cytoplasm, and the heavily granular cytoplasm. Therefore, her records of *"E. tenella"*, *"E. mitis"*, and *"E. acervulina"* from California quail should be regarded as erroneous, as should *E. maxima*, listed from California quail by Pellerdy (18), but for which I can find no original published report.

California quail were introduced into the Okanagan Valley from stock probably brought from California. It may be that the *Eimeria* species found in this study are the same as some of those reported by Herman and co-workers (4-8); these may have been brought into the area with the quail. However,

despite finding and recognizing at least five morphologically different types of oocysts, and despite unsuccessful attempts at transmitting four of these five species to domestic chickens, Herman or co-workers have not yet described or named any of the species they recognized. It is therefore impossible to compare my material with theirs.

Taking into account the unsuccessful attempts at cross transmission of oocysts of *Eimeria* from wild California quail to domestic chickens and Japanese quail, and noting the differences between the species (*Eimeria* species "A" and "B") and other species reported from Galliformes, I believe that the species reported herein are new and heretofore undescribed.

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